

## Fast K2/SPICE analysis using LDTD-TripleTOF™ 5600 System

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## Overview

- Fast screening and quantification method for K2/SPICE metabolites analysis in urine.
- The evaluation performed on JWH-018 and JWH-073 metabolites (**Figure 1**).
- A liquid-liquid extraction procedure is used to prepare the sample.
- Sample-to-sample run time of 7 seconds.
- Calibration range for High resolution screening: 2 to 2000 ng/ml with  $r^2 > 0.99$ .
- Calibration range for high sensitivity quantification method: 0.1 to 2000 ng/ml with  $r^2 > 0.99$ .



Figure 2 LDTD- AB Sciex TripleTOF™ 5600

## Introduction

K2/Spice are a class of chemical products known as synthetic cannabinoid drugs which, when consumed, mimics the effects of cannabis. Several US states have already made them illegal under state law and as of March 1, 2011, five cannabinoids, JWH-018, JWH-073, CP-47,497, JWH-200, and cannabicyclohexanol are now illegal in the US. Spice does not cause a positive drug test using traditional GC-MS-screening with library search, multi-target screening by LC-MS/MS, or immunological screening procedures. Moreover, chemists are developing new K2/Spice drugs which need to be identified and quantified. A study has been conducted for the purpose of detecting the metabolites of JWH-018 and JWH-073 in urine, and both the conjugates with glucuronic acid and the hydroxylated drugs have been found.

Using the LDTD-TripleTOF™ 5600 System, we propose to develop a fast and quantitative screening method for the JWH-018 and JWH-073 metabolites in urine. With the versatility of the TripleTOF™ 5600 System, we are also developing a High Resolution MS/MS method to lower the limit of detection.

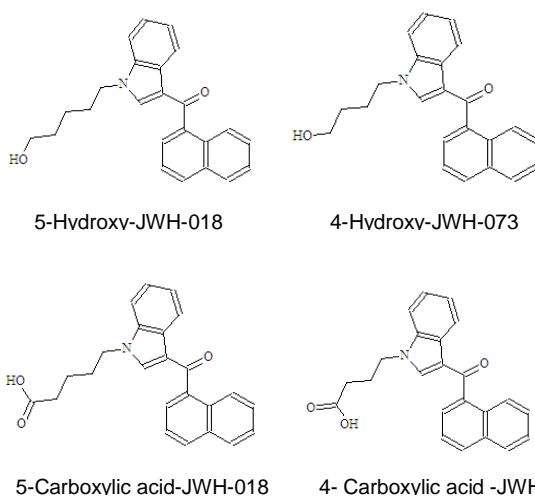


Figure 1 Chemical structures of JWH-073 and JWH-018 metabolites

## Instrumentation (Figure 2)

- Phytronix Technologies LDTD ion source (model S-960);
- AB SCIEX, TripleTOF™ 5600 System.

## Samples Preparation

### Liq-Liq extraction

- 100 µL urine sample
- 100 µL Internal standard solution (Dissolved in MeOH: HCl (0.5N) / 1:1 v/v)
  - o Vortex
- 300 µL Dichlorobutane
  - o Vortex and centrifuge (14000rpm/2min)
  - o Evaporate 200 µL of the organic phase
  - o Reconstitute with 20 µL of MeOH:Water (75:25 v/v) containing EDTA at 22.5µg/mL.
  - o Spot: 2 µL on 96 LazWell plate

### LDTD parameters

The analysis was performed using a 2 µL sample spotted into a 96 LazWell™ plate. The solvent was evaporated at room temperature. Carrier gas flow: 3 L/min. Laser desorption pattern: 2 seconds at 0 % of laser power, 2 seconds ramping the laser power up to 45 %, 3 seconds plateau at 45 % of laser power and laser power shut down to 0 % in 0.01 second.

## Results and Discussion

### Screening mode

For the drug screening, the LDTD-TripleTOF™ 5600 System was operated in scanning mode (High Resolution). The system scans in positive mode with a mass range between 100 to 900 amu with a mass window of 10 ppm. In this scanning mode, the tested K2/Spice metabolites are detected over a concentration range from 2 to 2000 ng/mL (Figure 3).

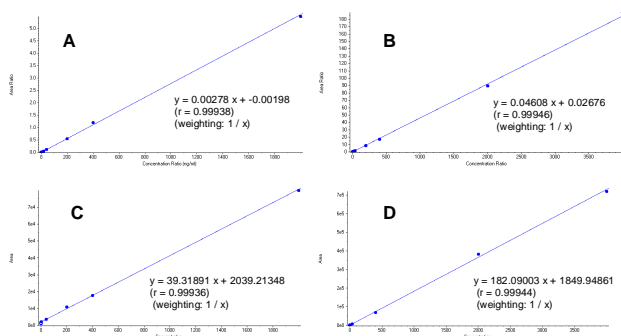


Figure 3 Standard curve in Scan mode. A) 5-OH-JWH-018, B) 4-OH-JWH-073, C) 5-COOH-JWH-018, D) 4-COOH-JWH-073.

Using the High Resolution mode, we are able to distinguish between 4-COOH-JWH-073 and 5-OH-JWH-018 even though they have the same nominal molecular mass (358.1 g/mol).

### High sensitivity quantification mode

If a more sensitive method is needed, we can operate the LDTD-TripleTOF™ 5600 System as a high resolution (HR) MS/MS system i.e Q1 filter for the metabolite m/z selected and high resolution TOF to get specific fragment from the collision cell. To do so, we extract mass from the product scan window at width of 10ppm. The same daughter ion was monitored for 4-OH-JWH-073 and 5-COOH-JWH-018 (HR-m/z 344.2 → 155.0050 amu 372.2 → 155.0050 amu). The daughters of 358.2, which enclose two different metabolites, are resolved by extracting at HR-m/z 230.1621 amu for 5-OH-JWH-018 and HR-m/z 230.1249 amu for 4-COOH-JWH-073.

In this HR-MS/MS configuration, we obtained an excellent limit of detection in urine extracts of 0.2 ng/mL for all metabolites and the linearity, evaluated up to 2000 ng/mL, was excellent with  $r^2 > 0.99$  (Figure 4).

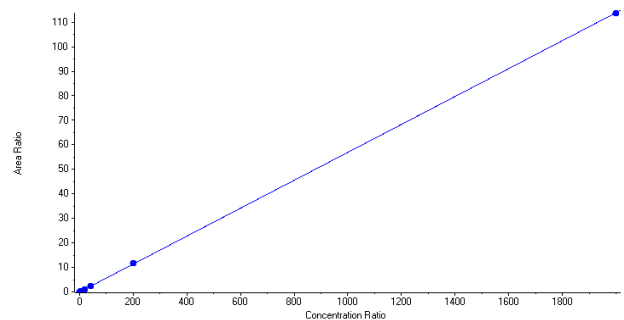


Figure 4 Standard curve of 5-Hydroxy-JWH-018 in High sensitivity quantification mode

## Conclusions

With the high resolution versatility of the new TripleTOF™ 5600 System and the high-throughput capability of the LDTD™ ion source we have developed an ultra-fast screening method running 1 sample every **7 seconds** with a lower limit of detection (2ng/mL) for JWH-018 and JWH-073 major metabolites. Alternatively, operating the system in high resolution MS/MS can achieve a detection limit of as low as 0.2 ng/mL.

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